Themed Issue: The Role of Microdialysis in Pharmacokinetics and Pharmacodynamics
Guest Editors - Markus Mueller and Ronald J. Sawchuk

Lung Microdialysis–A Powerful Tool for the Determination of Exogenous and Endogenous Compounds in the Lower Respiratory Tract (Mini-Review)

Submitted: May 18, 2005; Accepted: June 2, 2005; Published: October 22, 2005

Markus Zeitlinger,[1] Markus Müller,[1] and Christian Joukhadar[1]

[1]Department of Clinical Pharmacology, Division of Clinical Pharmacokinetics; Medical University of Vienna, Austria

Abstract

In vivo measurement of concentrations of drugs and endogenous substances at the site of action has become a primary focus of research. In this context the minimal invasive microdialysis (MD) technique has been increasingly employed for the determination of pharmacokinetics in lung. Although lung MD is frequently employed to investigate various drugs and endogenous substances, the majority of lung MD studies were performed to determine the pharmacokinetic profile of antimicrobials that can be related to the importance of respiratory tract infections. For the lower respiratory tract various methods, such as surgical collection of whole lung tissue and bonchoalveolar lavage (BAL), are currently available for the determination of pharmacokinetics of antimicrobials. Head-to-head comparison of pharmacokinetics of antibiotics in lung revealed high differences between MD and conventional methods. MD might be regarded as a more advantageous approach because of its higher anatomical resolution and the ability to obtain dynamic time-vs-concentration profiles within one subject. However, due to ethical objections lung MD is limited to animals or patients undergoing elective thoracic surgery. From these studies it was speculated that the concentrations in healthy lung tissue may be predicted reasonably by the measurement of concentrations in skeletal muscle tissue. However, until now this was only demonstrated for β-lactam antibiotics and needs to be confirmed for other classes of antimicrobials. In conclusion, the present review shows that MD is a promising method for the determination of antimicrobials in the lung, but might also be applicable for measuring a wide range of other drugs and for the investigation of metabolism in the lower respiratory tract.

Keywords: lung, microdialysis, pharmacokinetic, antibiotic

Corresponding Author: Markus Zeitlinger, Department of Clinical Pharmacology, Division of Clinical Pharmacokinetics, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria. Tel: +43-1-40400-2981; Fax: +43-1-40400-2998; E-mail: markus.zeitlinger@meduniwien.ac.at

Introduction

When the United States Food and Drug Administration (FDA) set up its first standards for approving and testing antimicrobial agents, it did not foresee the possibility that drugs do not act in the plasma compartment. Meanwhile, this regulatory authority and the European Agency for the Evaluation of Medicinal Products (EMEA) have recognized that plasma concentrations of antimicrobial agents are of less relevance and thus they focused their recent attention on tissue concentrations. Tissue levels of antimicrobials are considered much more important for the prediction of clinical and microbial efficacy. Thus, in vivo determination of pharmacokinetic profiles of drugs and endogenous substances at the site of action has become a major focus in research by industry and academia. The measurement of target site concentrations in lung remains an ongoing challenge because of the protected anatomical position and its high vulnerability. In this context the minimal invasive microdialysis (MD) technique, a well-established method for the determination of pharmacokinetics of a wide range of substances in various tissues, has been increasingly employed during the past few years.[1,2] Data from human and animal studies have highlighted the value of this new method for the description of continuous time-vs-concentration profiles of drugs and endogenous substances in the interstitial space fluid of the lung. The present work sets out to review the current applications of MD in human and animal lung in vivo and compares the advantages and disadvantages of MD and other methods for the investigation of drug pharmacokinetics in lung.

Technical Challenges of Lung MD

The principles of MD for clinical and animal studies in various human tissues have been described and reviewed in detail previously.[1-5] Briefly, the basic principle of MD is to perfuse the implanted MD probe with a perfusate, ie, a physiologic liquid at a very slow rate (Figure 1). Substances present in the interstitial space fluid of the investigated site can diffuse into the perfusate through a semipermeable membrane and appear in the dialysate at an individually determined percentage called "recovery." Afterward the concentration in the dialysate is chemically analyzed and by

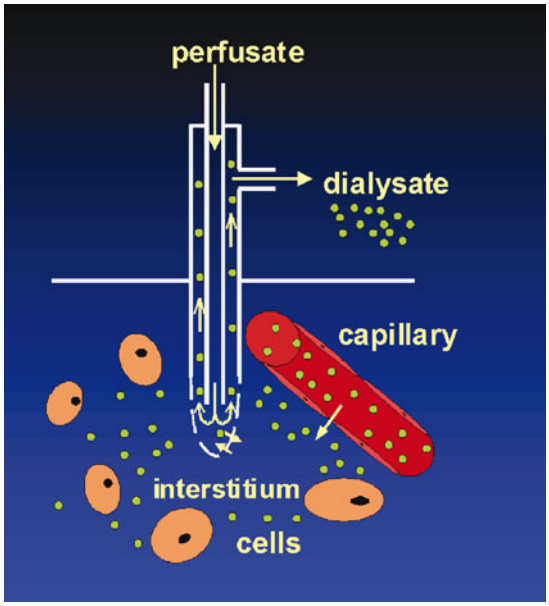

Figure 1. Principles of MD: the implanted MD probe is perfused with the perfusate, ie, a physiologic liquid at a very slow rate. Substances present in the interstitial space fluid of the investigated site can diffuse into the perfusate through a semipermeable membrane at the tip of the MD probe and appear in the dialysate. Afterward the concentration in the dialysate is chemically analyzed and the true concentration in the interstitial space fluid can be calculated.

use of the respective recovery-value the true concentration in the interstitial space fluid can be calculated.

Although MD is a widely used technique for the measurement of drugs and metabolic substances in many different tissues, until now it has only been employed in about a dozen studies in lung. This might be related to the wide range of alternative techniques available for the determination of concentrations in lung tissue. Some of these techniques, such as collection of sputum or bronchial secretions, are considered noninvasive and can be performed in healthy volunteers.[6,7] In contrast, implementation of lung MD is not possible in healthy volunteers due to ethical objections. As described for animal studies, implantation of MD probes through the chest wall may lead to pneumothorax and collapse of the lung.[8] To avoid the risk of pneumothorax, the performance of thoracotomy is inevitable (Figure 2); thereby limiting MD experiments in the human lung to elective thoracic surgery.[9-11] However, if the MD probe is properly implanted under direct vision during conventionally performed lung surgery, no adverse events or clinical complications related to MD have been reported.[9-11]Lung MD in clinical studies was performed in spontaneously breathing patients after the end of the surgical procedures. In these studies flexible custom-made MD probes (Metalant AB, Stockholm, Sweden) with a total length of ~50 cm to allow for pulmonal movement due to respiration were

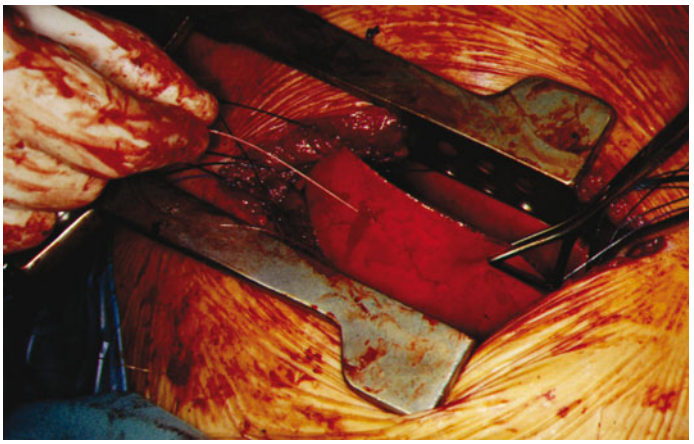

Figure 2. Implantation of an MD probe into the lung during elective thoracic surgery. In clinical studies MD was started in spontaneously breathing patients after closing of the chest wall.

employed.[10] The tip of the probes was made of a 0.6 × 50-mm polyether sulphone membrane in order to achieve high recovery rates (43% to 78%, dependent on the flow rate of the perfusion media), which allows for accurate calculation of in vivo concentrations. Although the probe is also in contact with alveoli, membrane exchange will not take place with alveolar air, but only with the fluid phase.[10] Therefore, the concentrations in the samples will exclusively represent concentrations in the interstitial space and the alveolar lining fluid.

Shorter MD probes have been shown to be sufficient for small animals. In animal models performing lung MD, usually CMA 10[8,12,13] or CMA 20 probes[12-14] (both CMA Microdialysis, Solna, Sweden) with membrane lengths of 10 to 16 mm were used. Some animal studies performed lung MD during cardiopulmonary bypass with cardiac arrest. Due to the limited movement of the lung in this condition short MD probes might be also be sufficient for larger animals.[14] However, employment of shorter MD probes has been shown to lead to substantial reduction of recovery rates, which might result in unreliable data.[8]

Current Focus of Lung MD

The first study reporting lung MD was published in 1991 and investigated the pharmacokinetics of theophylline in rats.[15] However, the majority of lung MD studies have been performed for the determination of time-vs-concentration profiles of antimicrobial agents. Lung concentrations of meropenem, piperacillin and cefpirome, cefpodoxime, cefaclor, faropenem, tobramycin, and gentamycin have been investigated by MD in humans or animals.[9-12,16-18]

The increasing interest in the pharmacokinetics of antimicrobial drugs in the lung might be related to the fact that over 60% of infections at intensive care units are infections of the lower respiratory tract.[19] In addition, community-acquired

pneumonia has been identified as the most common cause of death in the western world due to infectious diseases.[20] Failure of antibiotic therapy may be linked to bacterial resistance against antibiotics or impaired penetration of antimicrobial agents into the site of infection.[21-23] In contrast to other drugs, the importance of pharmacokinetic-pharmacodynamic relationships for antimicrobial and clinical efficacy has been widely documented for antibiotics.[24,25] In addition, pneumonia is an infectious disease where a close correlation between the clinical outcome and pharmacokinetic-pharmacodynamic surrogate parameters was demonstrated for humans.[26] However, target site concentrations may be substantially lower than plasma concentrations and, therefore, regulatory authorities like the EMEA and the FDA recommend measuring target site concentrations of antimicrobials rather than plasma concentrations to predict outcome of anti-infective therapy.[27,28] Since MD is a well-established method for the measurement of free antibiotic concentrations at the site of infection,[4,5] it is not surprising that lung MD has mainly been performed to comply with the scientific and commercial interest in the pharmacokinetics of antimicrobials in the lower respiratory tract.

Methodical Considerations for Determining Concentrations of Antimicrobials in Lung

Because of the anatomy and the histological structure of the lung, concentrations of drugs may vary within the organ.[29,30] Exact attribution of the measured concentrations to the different compartments in lung has been considered as a prerequisite for the interpretation of pharmacokinetics of antimicrobial agents in lung.[29] Early techniques investigating the ability of antibiotics to penetrate into the lower respiratory tract include sampling of sputum,[31] respiratory secretions,[32-35] pleural fluid[36] and surgical collection of whole lung tissue[33,37] and bronchial mucosa.[31,37] Since the first application of bonchoalveolar lavage (BAL) in the late 1960s[38] the technique has been employed in numerous studies, and sampling of epithelial lining fluid (ELF)[39-41] and alveolar macrophages[40,42] by BAL is currently most often employed for pharmacokinetic research in lung. In addition to these techniques, imaging procedures like planar gamma scintigraphy and positron emission tomography have been applied for the determination of distribution patterns in lung.[43,44] However, several major methodical problems limit the interpretation of data derived from all these methods:

- *Finding a correlation between concentrations in the sample and the concentration at the target site.* To estimate the dilution rate due to the instilled fluid during BAL, endogenous markers like urea are commonly used. However, a dilution of more than 100-fold is frequent and, therefore, calculation of the true concentration may sometimes be imprecise.[41,45] Several authors mentioned, that a potential source of error of investigating antibiotic concentrations by BAL may be the antibiotic release from alveolar macrophages in the sample.[30,46,47] This may lead to overestimation of actual active extracellular concentrations in the ELF.
- *Correlating pharmacokinetic data to a specific anatomic site.* Concentrations obtained with BAL represent average concentrations over a large segment of the lung, since saline may distribute freely throughout the bronchial system during lavage. However, significant differences of antibiotic concentrations have been described between bronchial secretions and acini in the lower respiratory tract.[48]
- *Correlating pharmacokinetic data to a histological site.* For biopsy with consecutively tissue homogenization admixture of concentrations of blood, and intracellular and extracellular contents is unavoidable. Analogically, also imaging procedures like scintigraphy and positron emission tomography are not able to discriminate between different histological compartments.
- *Use of longitudinal data in order to obtain time-concentration-profiles.* Tissue biopsy lacks the ability to monitor dynamic changes in concentrations of drugs in a single subject and time-concentration profiles can only be derived by use of longitudinal pharmacokinetic data. Although BAL can be performed repeatedly in one subject, the standard lavage technique involves instillation of saline into the lung with consecutive aspiration of the lavage fluid. Since each episode of lavage alters the composition of ELF, frequent sampling at short time intervals would falsify the results thereby limiting the ability of this technique to determine dynamic time-concentration-profiles.[45]
- *Discrimination between free, ie, microbiologically active and bound fraction.* Various studies have demonstrated that only the unbound fraction of an antibiotic becomes microbiologically active.[49] Concentrations of antimicrobials obtained from homogenized tissues or by use of imaging techniques are not only an average of intracellular and extracellular concentrations, but include also those fractions of an antibiotic that are bound to interstitial proteins or intracellular and intercellular membrane structures.[18]

In contrast, MD enables the continuous measurement of the unbound drug concentrations at an exact histological site, ie, the interstitial space fluid. However, determination of

antibiotic concentrations in lung by MD comprises also some limitations. This can be attributed to the fact that infections in the lower respiratory tract may be located in different compartments. In pneumonia, infections (typically caused by *Streptococcus pneumoniae*) are rather located in the alveolar region or, after parenchymal consolidation, in the interstitium of lung. Therefore, antimicrobials must reach the interstitial space and the alveolar lining fluid to become active. In contrast, for endobronchial infections (typically caused by *Hemophilus influenzae* and *Moraxella catarrhalis*) the antibiotic should rather concentrate in the bronchial wall and the bronchial lumen.[29,46] For the more seldom infections caused by intracellular pathogens (*Mycoplasma* spp, *Chlamydia* spp, and *Legionella* spp) the antibiotic should reach high concentrations in alveolar cells and phagocytic cells of the lung and ELF.[29,50] To compare the pharmacokinetics of antibiotics in different compartments of the respiratory tract, concentrations of meropenem and piperacillin, 2 β-lactam antibiotics, obtained by various methods will be discussed.

Comparing Pharmacokinetic Data Obtained by MD to Data by Other Methods

Maximum concentration (C_{max}) obtained by MD and other methods from the human respiratory tract are presented in Table 1. However, none of the studies performing lung MD has simultaneously employed other methods for the determination of antimicrobial concentrations in the lower respiratory tract. Therefore, comparison of pharmacokinetic data from different studies can only be done with caution, since highly variable groups of patients (healthy subjects and septic patients) and different dosing regimes (single dose and steady-state) have been investigated. To demonstrate acceptable comparability within the discussed studies, C_{max} values of plasma have been included in the table and show almost identical plasma pharmacokinetics in nearly all presented studies.

In general, determination of meropenem and piperacillin by lung MD resulted in higher concentrations than corresponding C_{max} values obtained with other methods. However, this might be attributed to the fact that apart from MD studies most C_{max} values are determined from only 1 to 6 time points, which easily could miss the actual maximal concentration. In addition to the limited sampling time points, the lower concentrations of meropenem and piperacillin in lung and bronchial mucosa from biopsies can be explained by the fact that whole-tissue concentrations represent an average of concentrations of different compartments. Since the cellular penetration of beta-lactam antibiotics is poor, the concentrations in the interstitial fluid can be expected to be higher than the observed concentration in biopsy homogenates.[52] In contrast, investigations of other classes of antibiotics like fluoroquinolons or macrolides, which accumulate in cells, might result in higher concentrations in biopsy homogenates and ELF than in the interstitial space fluid of lung.[40,53,54]

Concentrations of meropenem determined in ELF are much more congruent with pharmacokinetic data obtained by MD than concentrations of piperacillin.[41,51] However, while meropenem was determined in ELF at 6 time points, piperacillin was measured only 5 hours after application of the drug, and the actual C_{max} value of piperacillin in ELF was most probably not detected.

Prediction of Target Site Concentrations in Lung by MD in Other Tissues

As previously discussed, lung MD is more challenging than in peripheral tissues and is limited to animals or restricted patient populations. Therefore, clinicians ask for "surrogate tissues," which allow for prediction of concentrations in the lung. Recently, 2 animal studies have been designed to compare the concentrations of the antibiotics cefpodoxime and cefaclor in peripheral tissue and lung by use of MD.[12,16] Due to the similar content of water in lung and skeletal muscle tissue, muscle tissue can be considered more suitable to represent conditions in the lung than subcutaneous adipose tissue.[55] Therefore, these studies simultaneously employed MD in skeletal muscle and lung tissue of rats in order to determine whether the more easily accessible skeletal muscle could be taken as a surrogate for concentrations in the lung. Both studies found that unbound concentrations in the interstitial space fluid of skeletal muscle and lung tissue are descriptively identical (Table 2) and suggested that concentrations in skeletal muscle may be reasonable predictors for concentrations in lung.

Based on the fact that cefpodoxime identically penetrated into skeletal muscle and lung tissue of rats, lung penetration of cefpodoxime in humans was estimated by employing MD in skeletal muscle of healthy volunteers.[12] However, this approach seems only justified if the similar penetration into lung and skeletal muscle observed in rats may be translated to humans. Two different studies performing lung MD in patients undergoing thoracotomy determined simultaneously concentrations of piperacillin or meropenem in skeletal muscle tissue.[9,11] Another study investigating concentrations of cefpirome in lung tissue appears, due to identical dosing regimes, comparable to a previous study performing MD in skeletal muscle tissue.[10,56] Similar area under the concentration time curve (AUC) and C_{max} values for lung and skeletal muscle tissue were found in these studies and indicated that also in humans the pharmacokinetics in lung tissue may be reasonably predicted by the concentrations in skeletal muscle tissue (Table 2).

For piperacillin and meropenem these results may be unexpected, because the MD probes were inserted into

Table 1. C_{max} of Meropenem and Piperacillin in Lung Tissue and Plasma Obtained by Different Techniques After Single Dose (SD) or at Steady-State (SS)*

Antibiotic	Method	C_{max} Lung, mg/L	Dose, g	C_{max} Plasma, mg/L	No. Sampling Time Points	No. Patient Groups to Pool Data	Authors
Meropenem	Microdialysis	11.4 ± 10.9	1 (SS)	47.3	18	1	Tomaselli et al 2004[11]
	Lung biopsy	3.9 (0.2 to 8.2)	1 (SD)	20	3	3	Byl et al 1999[37]
	Bronchial muscosa biopsy	6.6 (3.0 to 13.3)	1 (SD)	20	3	3	Byl et al 1999[37]
	BAL (ELF)	7.1 ± 2.9	1 (SD)	26.0	6	6	Allegranzi et al 2000[41]
	Bronchial secretion	0.53 ± 0.41	1 (SD)	59.8	3	3	Bergogne-Berezin et al 1994[35]
Piperacillin	Microdialysis	176 ± 105	4 (SS)	326	12	1	Tomaselli et al 2003[9]
	Bronchial muscosa biopsy	55.2 ± 12.8	4 (SS)	196.3	1	1	Marlin et al 1981[31]
	BAL (ELF)	13.6 ± 9.4	4 (SS)	ND	1	1	Boselli et al 2004[51]
	Bronchial secretion	29.3 ± 25.1	4 (SS)	184.8	6	1	Jehl et al 1994[34]
	Bronchial secretion	31.4 ± 11.3	4 (SS)	196.3	1	1	Marlin et al 1981[31]
	Sputum	13.0 ± 6.7	4 (SS)	347.9	3	1	Marlin et al 1981[31]

C_{max}, Maximum concentration; *BAL, b*ronchoalveolar lavage; *ELF,* epithelial lining fluid; ND, no data.
*The number of sampling time points to determine C_{max} is shown. In addition, the number of patient groups to obtain longitudinal pharmacokinetic data from different individuals is indicated.

pneumonic lung tissue, while healthy skeletal muscle tissue was used for comparison. However, although recent studies indicate that tissue penetration may be considerably lower in patients as compared with healthy subjects, this difference was rather attributed to septic conditions and intensive care measures than to local inflammation.[21,22,56,57] Since sepsis was diagnosed in both studies, it is not surprising that AUC values in lung and skeletal muscle tissue were significantly lower than in plasma, while no significant difference between lung and skeletal muscle tissue was detected.

The presented studies indicate that penetration of antimicrobial agents into lung and peripheral tissue might be altered to a similar extent, and skeletal muscle tissue might represent a worthy surrogate to predict concentrations of antimicrobials in lung. However, until now this has only been shown for β-lactams and cannot be transferred to other antimicrobial classes without further investigations. This is determined by looking at pathologies like cystic fibrosis with progressive destruction of airway structures and abundant production of thick purulent bronchial secretions have been described to

Table 2. Comparison of AUC and C_{max} Values of Different β-lactam Antibiotics in Lung Tissue and Skeletal Muscle Determined by MD in Clinical Studies*

	AUC_{Lung} (mg*h/L)	$C_{max\ Lung}$ (mg/L)	AUC_{Muscle} (mg*h/L)	$C_{max\ Muscle}$ (mg/L)	AUC_{Plasma} (mg*h/L)	$C_{max\ Plasma}$ (mg/L)
Cefaclor[16] (50 mg/kg, rat)	25.0 ± 12.9	29.2 ± 10.4	25.8 ± 9.6	28 ± 5.4	169.7 ± 97.9	270.2 ± 62.1
Cefpirome (2 g)	174 ± 15[10]	74 ± 24[10]	163 ± 12[31]	62 ± 4[31]	261 ± 24[10] 267 ± 19[31]	175 ± 18[10] 164 ± 14[31]
Meropenem[11] (1 g, SS, septic patients)	36.2 ± 17.9	11.4 ± 10.9	44.6 ± 30	26.2 ± 25.2	95.4 ± 46.6	47.3 ± 21.0
Piperacillin[9] (4 g, SS, septic patients)	288 ± 167	176 ± 105	197 ± 122	76 ± 22	470 ± 142	326 ± 61

AUC, Area under the concentration time curve; C_{max}, maximum concentration; *MD,* microdialysis; *SS,* steady-state.
*Pharmacokinetic data obtained from plasma are also presented.

influence penetration of antibiotics into the lung.[29,58,59] Without further investigation it is doubtful that concentrations in skeletal muscle tissue can predict the altered permeability of antimicrobials into lung in such conditions.[58,59]

Determination of Antibiotic Concentrations in the ELF by MD

Bacterial adherence in the bronchial mucosa is often the first step of pulmonary involvement of respiratory tract infections. High antimicrobial concentrations in the mucosa and ELF might inhibit bacterial adhesion and subsequent tissue invasion.[29,46] Since the microbiologically active concentration of an antibiotic in the bronchial wall and in the ELF is the result of a complex and dynamic process,[60] usually a combination of bronchial mucosa biopsy and sampling of BAL is performed for the determination of bronchial pharmacokinetics.[61] The general limitations of BAL, especially its poor anatomical resolution and inability to discriminate between antimicrobially active and inactive drug, have been discussed in a previous section of the review. In contract to BAL, MD allows for the dynamic determination of the extracellular, nonprotein-bound concentration of an antibiotic and, therefore, intrabronchial MD seems to be an interesting alternative for determination of antimicrobials in the ELF.

Until now, only one study used MD to measure the concentration of the aminoglycosides tobramycin and gentamycin in ELF in vivo.[17] MD probes were implanted into the bronchial system of rats via tracheotomy. The mean penetration of tobramycin and gentamycin indicated by the $AUC_{tissue}/AUC_{plasma}$ ratio (0.36 and 0.56, respectively) was comparable to results previously determined by BAL (0.45 and 0.67).[39] The similar results obtained by both methods can be attributed to the fact that aminoglycosides are known to exclusively distribute in the extracellular space fluid. Therefore, concentrations obtained by BAL have not been diverted by effusion of intracellular amounts of drug as known for macrolides of fluoroquinolons.[53,62,63]

Rare Applications of Lung MD

Several studies employed MD to monitor non-anti-infective drugs, endogenous substances, and pathophysiologic processes in the lung.

Theophylline

Recently, the relationship between the concentration of theophylline and both its adverse and beneficial bronchodilatory effects has been demonstrated.[64,65] However, similar to antibiotics, most data were obtained by determination of concentrations of theophylline in serum rather than at the target site in the lung.[66] Theophylline concentrations in lung have been investigated in 2 MD studies.[8,15] The first study set out to determine changes in the recovery of theophylline in lung and blood of rats during the MD experiment. The second study investigated whether the time-concentration course in plasma reflects the concentration of theophylline in the lung in rats and horses. Variation of recovery with time was considerably lower for lung tissue as compared with blood, suggesting that determination of the recovery rate at the beginning and the end of the experiment might sufficiently describe the recovery of theophylline in lung.[15] After intravenous application of theophylline to rats and horses the free concentration in the lung was significantly lower than corresponding free concentrations in serum in both species.[8] This was attributed to the metabolic degeneration of theophylline in the lung.[67]

Determination of Metabolic Processes in Lung

Until now, the majority of MD studies investigating metabolic processes in vivo have been limited to brain research.[68-70] A recent MD study set out to evaluate changes of concentrations of metabolites in the lung during ischemic reperfusion injury.[14] Ischemic reperfusion injury of lung tissue after cardiac arrest occurs in virtually all patients undergoing cardiopulmonary bypass.[71] The potential of cariporide, a substance that has been shown to reduce acidosis due to ischemia,[72] to protect the lung from injury during cardiopulmonary bypass was evaluated.[14] Lung MD was used to measure concentrations of glycerol, ie, a marker reflecting the breakdown of the membrane phospholipids, in lung tissue of pigs undergoing cardiac arrest.[68,69] Although no difference in ischemia/reperfusion injury between cariporide and placebo was detected, MD was suggested to be a promising tool for in vivo studies of metabolic processes in the lung.

Investigation of Pathophysiologic Changes in Lung

Mucociliary transport and clearance of particles and bacteria from the airway surface represents the first line of defense against infections of the lower respiratory tract. Due to genetic disorders such as primary ciliary dyskinesia and cystic fibrosis this mechanical defense mechanism may be reduced, which results in higher incidence of respiratory infections in these patients.[73,74] Recently, lung MD was employed for the determination of mucociliary transport in normal and cystic fibrosis (CF) mice.[13] An MD probe was implanted into the lumen of the trachea and a small quantity of dye was placed on the surface of a distal smaller bronchus at a known distance to the tip of the MD probe. The rate of bronchial mucociliary transport was calculated as the time from dye deposition to its first detection in the MD samples. Although in this study no significant difference in mucociliary transport between normal and CF mice could

be detected, this seems an interesting method for determination of mucociliary transport rates.

SUMMARY

Drug concentrations at the target site have been accepted to be more predictive for the effect of a drug than plasma concentrations. Currently, various methods for the determination of pharmacokinetics of antibiotics in the lower respiratory tract are available. Head-to-head comparison of maximal concentrations of antibiotics in the lower respiratory tract revealed high differences between methods.

MD was a more advantageous approach compared with conventional methods because of its higher anatomical resolution and the ability to obtain dynamic time-vs-concentration profiles within one subject. Additionally, in clinical studies MD proved to be a feasible and safe method to measure concentrations in human lung in vivo. However, depending on the pathogen the infection takes place at different compartments of the lower respiratory tract. Since concentrations might significantly differ between these compartments, only the combination of different methods seems able to appropriately describe the pharmacokinetics at different sites of the respiratory tract.

In conclusion, MD has been shown to be a useful tool for determination of free extracellular concentration in both the interstitial space fluid of lung and in the ELF. Alternative applications of lung MD indicate that MD might be also used for the measurement of a wide range of substances and might be a suitable method for the investigation of metabolism and pathophysiologic processes in the lung.

REFERENCES

1. Muller M. Microdialysis in clinical drug delivery studies. *Adv Drug Deliv Rev*. 2000;45:255-269.

2. de la Pena A, Liu P, Derendorf H. Microdialysis in peripheral tissues. *Adv Drug Deliv Rev*. 2000;45:189-216.

3. Ungerstedt U. Microdialysis—principles and applications for studies in animals and man. *J Intern Med*. 1991;230:365-373.

4. Muller M, Haag O, Burgdorff T, et al. Characterization of peripheral-compartment kinetics of antibiotics by in vivo microdialysis in humans. *Antimicrob Agents Chemother*. 1996;40:2703-2709.

5. Joukhadar C, Derendorf H, Muller M. Microdialysis. A novel tool for clinical studies of anti-infective agents *Eur J Clin Pharmacol*. 2001;57:211-219.

6. Chalmers GW, Thomson L, Macleod KJ, et al. Endothelin-1 levels in induced sputum samples from asthmatic and normal subjects. *Thorax*. 1997;52:625-627.

7. Conte JEJr, Golden JA, Kipps J, McIver M, Zurlinden E. Intrapulmonary pharmacokinetics and pharmacodynamics of itraconazole and 14-hydroxyitraconazole at steady state. *Antimicrob Agents Chemother*. 2004;48:3823-3827.

8. Ingvast-Larsson C, Appelgren LE, Nyman G. Distribution studies of theophylline: microdialysis in rat and horse and whole body autoradiography in rat. *J Vet Pharmacol Ther*. 1992;15:386-394.

9. Tomaselli F, Dittrich P, Maier A, et al. Penetration of piperacillin and tazobactam into pneumonic human lung tissue measured by in vivo microdialysis. *Br J Clin Pharmacol*. 2003;55:620-624.

10. Herkner H, Muller MR, Kreischitz N, et al. Closed-chest microdialysis to measure antibiotic penetration into human lung tissue. *Am J Respir Crit Care Med*. 2002;165:273-276.

11. Tomaselli F, Maier A, Matzi V, Smolle-Juttner FM, Dittrich P. Penetration of meropenem into pneumonic human lung tissue as measured by in vivo microdialysis. *Antimicrob Agents Chemother*. 2004;48:2228-2232.

12. Liu P, Muller M, Grant M, Webb AI, Obermann B, Derendorf H. Interstitial tissue concentrations of cefpodoxime. *J Antimicrob Chemother*. 2002;50:19-22.

13. Grubb BR, Jones JH, Boucher RC. Mucociliary transport determined by in vivo microdialysis in the airways of normal and CF mice. *Am J Physiol Lung Cell Mol Physiol*. 2004 ;286:L588-L595.

14. Eichler W, Bechtel MJ, Klaus S, et al. Na /H+ exchange inhibitor cariporide: effects on respiratory dysfunction after cardiopulmonary bypass. *Perfusion*. 2004;19:33-40.

15. Larsson CI. The use of an "internal standard" for control of the recovery in microdialysis. *Life Sci*. 1991;49:PL73-PL78.

16. De La Pena A, Dalla Costa T, Talton JD, et al. Penetration of cefaclor into the interstitial space fluid of skeletal muscle and lung tissue in rats. *Pharm Res*. 2001;18:1310-1314.

17. Eisenberg EJ, Conzentino P, Eickhoff WM, Cundy KC. Pharmacokinetic measurement of drugs in lung epithelial lining fluid by microdialysis: aminoglycoside antibiotics in rat bronchi. *J Pharmacol Toxicol Methods*. 1993;29:93-98.

18. DeGuchi Y, Terasaki T, Yamada H, Tsuji A. An application of microdialysis to drug tissue distribution study: in vivo evidence for free-ligand hypothesis and tissue binding of beta-lactam antibiotics in interstitial fluids. *J Pharmacobiodyn*. 1992;15:79-89.

19. Vincent JL, Bihari DJ, Suter PM, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. *JAMA*. 1995;274:639-644.

20. Bartlett JG, Mundy LM. Community-acquired pneumonia. *N Engl J Med*. 1995;333:1618-1624.

21. Zeitlinger MA, Dehghanyar P, Mayer BX, et al. Relevance of soft-tissue penetration by levofloxacin for target site bacteria killing in patients with sepsis. *Antimicrob Agents Chemother*. 2003;47:3548-3553.

22. Joukhadar C, Frossard M, Mayer BX, et al. Impaired target site penetration of beta-lactams may account for therapeutic failure in patients with septic shock. *Crit Care Med*. 2001;29:385-391.

23. Lambert HP. Clinical significance of tissue penetration of antibiotics in the respiratory tract. *Scand J Infect Dis Suppl*. 1978;14:262-266.

24. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men *Clin Infect Dis*. 1998;26:1-12.

25. Drusano GL, Craig WA. Relevance of pharmacokinetics and pharmacodynamics in the selection of antibiotics for respiratory tract infections *J Chemother*. 1997;9:38-44.

26. Schentag JJ. Correlation of pharmacokinetic parameters to efficacy of antibiotics: relationships between serum concentrations, MIC values,

and bacterial eradication in patients with gram-negative pneumonia. *Scand J Infect Dis Suppl*. 1990;74:218-234.

27. EMEA. Points to consider on pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products. Available at: http://www.emea.eu.int/pdfs/human/ewp/265599en.pdf. Accessed October 11, 2005.

28. FDA. Guidance for Industry. Developing antimicrobial drugs—general considerations for clinical trials. Available at: http://www.fda.gov/cder/guidance/2580dft.pdf. Accessed October 11, 2005.

29. Bergogne-Berezin E. New concepts in the pulmonary disposition of antibiotics. *Pulm Pharmacol*. 1995;8:65-81.

30. Gail DB, Lenfant CJ. Cells of the lung: biology and clinical implications. *Am Rev Respir Dis*. 1983;127:366-387.

31. Marlin GE, Burgess KR, Burgoyne J, Funnell GR, Guinness MD. Penetration of piperacillin into bronchial mucosa and sputum. *Thorax*. 1981;36:774-780.

32. Klastersky J, Thys JP, Mombelli G. Comparative studies of intermittent and continuous administration of aminoglycosides in the treatment of bronchopulmonary infections due to gram-negative bacteria. *Rev Infect Dis*. 1981;3:74-83.

33. Brun Y, Forey F, Gamondes JP, Tebib A, Brune J, Fleurette J. Levels of erythromycin in pulmonary tissue and bronchial mucus compared to those of amoxycillin. *J Antimicrob Chemother*. 1981;8:459-466.

34. Jehl F, Muller-Serieys C, de Larminat V, Monteil H, Bergogne-Berezin E. Penetration of piperacillin-tazobactam into bronchial secretions after multiple doses to intensive care patients. *Antimicrob Agents Chemother*. 1994;38:2780-2784.

35. Bergogne-Berezin E, Muller-Serieys C, Aubier M, Dombret MC. Concentration of meropenem in serum and in bronchial secretions in patients undergoing fibreoptic bronchoscopy. *Eur J Clin Pharmacol*. 1994;46:87-88. Erratum in.Eur J Clin Pharmacol. 1994;46:282.

36. Cole DR, Pung J. Penetration of cefazolin into pleural fluid. *Antimicrob Agents Chemother*. 1977;11:1033-1035.

37. Byl B, Jacobs F, Roucloux I, de Franquen P, Cappello M, Thys JP. Penetration of meropenem in lung, bronchial mucosa, and pleural tissues. *Antimicrob Agents Chemother*. 1999; 43:681-682.

38. Reynolds HY. Use of bronchoalveolar lavage in humans—past necessity and future imperative. *Lung*. 2000;178:271-293.

39. Valcke Y, Pauwels R, Van der Straeten M. The penetration of aminoglycosides into the alveolar lining fluid of rats. The effect of airway inflammation. *Am Rev Respir Dis*. 1990;142:1099-1103.

40. Panteix G, Harf R, Desnottes JF, et al. Accumulation of pefloxacin in the lower respiratory tract demonstrated by bronchoalveolar lavage. *J Antimicrob Chemother*. 1994;33:979-985.

41. Allegranzi B, Cazzadori A, Di Perri G, et al. Concentrations of single-dose meropenem (1 g iv) in bronchoalveolar lavage and epithelial lining fluid. *J Antimicrob Chemother*. 2000;46:319-322.

42. Harf R, Panteix G, Desnottes JF, Diallo N, Leclercq M. Spiramycin uptake by alveolar macrophages. *J Antimicrob Chemother*. 1988;22:135-140.

43. Le Conte P, Potel G, Peltier P, et al. Lung distribution and pharmacokinetics of aerosolized tobramycin. *Am Rev Respir Dis*. 1993;147:1279-1282.

44. Wollmer P, Pride NB, Rhodes CG, et al. Measurement of pulmonary erythromycin concentration in patients with lobar pneumonia by means of positron tomography. *Lancet*. 1982;320:1361-1364.

45. Allegranzi B. Concentrations of single-dose meropenem (1 g iv) in bronchoalveolar lavage and epithelial lining fluid. *J Antimicrob Chemother*. 2000;46:319-322.

46. Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: in vivo observations and clinical relevance. *Antimicrob Agents Chemother*. 1992;36:1176-1180.

47. Decre D, Bergogne-Berezin E. Pharmacokinetics of quinolones with special reference to the respiratory tree. *J Antimicrob Chemother*. 1993;31:331-343.

48. Braude AC, Hornstein A, Klein M, Vas S, Rebuck AS. Pulmonary disposition of tobramycin. *Am Rev Respir Dis*. 1983;127:563-565.

49. Kunin CM. Clinical significance of protein binding of the penicillins. *Ann N Y Acad Sci*. 1967;145:282-290.

50. Tulkens PM. Intracellular pharmacokinetics and localization of antibiotics as predictors of their efficacy against intraphagocytic infections. *Scand J Infect Dis Suppl*. 1990;74:209-217.

51. Boselli E, Breilh D, Cannesson M, et al. Steady-state plasma and intrapulmonary concentrations of piperacillin/tazobactam 4 g/0.5 g administered to critically ill patients with severe nosocomial pneumonia. *Intensive Care Med*. 2004;30:976-979.

52. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ. Antibiotic tissue penetration and its relevance: impact of tissue penetration on infection response. *Antimicrob Agents Chemother*. 1991;35:1953-1959.

53. Zeitlinger M, Graninger W, Joukhadar C. Pharmacokinetics of the macrolides azithromycin, clarithromycin and roxithromycin in plasma and tissue—are there significant differences? In: *Wien Klin Wochenschr* vol. 11-12a. 2004:18-24.

54. Rodvold KA, Gotfried MH, Danziger LH, Servi RJ. Intrapulmonary steady-state concentrations of clarithromycin and azithromycin in healthy adult volunteers. *Antimicrob Agents Chemother*. 1997;41:1399-1402.

55. Poulin P, Theil FP. A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. *J Pharm Sci*. 2000;89:16-35.

56. Joukhadar C, Klein N, Mayer BX, et al. Plasma and tissue pharmacokinetics of cefpirome in patients with sepsis. *Crit Care Med*. 2002;30:1478-1482.

57. Joukhadar C, Stass H, Muller-Zellenberg U, et al. Penetration of moxifloxacin into healthy and inflamed subcutaneous adipose tissues in humans. *Antimicrob Agents Chemother*. 2003;47:3099-3103.

58. LeBel M. Fluoroquinolones in the treatment of cystic fibrosis: a critical appraisal. *Eur J Clin Microbiol Infect Dis*. 1991;10:316-324.

59. Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. *Am Rev Respir Dis*. 1985;132:761-765.

60. Barza M, Cuchural G. General principles of antibiotic tissue penetration. *J Antimicrob Chemother*. 1985;15:59-75.

61. Muller-Serieys C, Bancal C, Dombret MC, et al. Penetration of cefpodoxime proxetil in lung parenchyma and epithelial lining fluid of noninfected patients. *Antimicrob Agents Chemother*. 1992;36:2099-2103.

62. Prokesch RC, Hand WL. Antibiotic entry into human polymorphonuclear leukocytes. *Antimicrob Agents Chemother*. 1982;21:373-380.

63. Forsgren A, Bellahsene A. Antibiotic accumulation in human polymorphonuclear leucocytes and lymphocytes. *Scand J Infect Dis Suppl*. 1985;44:16-23.

64. Peck CC, Nichols AI, Baker J, Lenert LL, Ezra D. Clinical pharmacodynamics of theophylline. *J Allergy Clin Immunol*. 1985;76:292-297.

65. Devarajan PV, Sule PN, Parmar DV. Comparative pharmacodynamic-pharmacokinetic correlation of oral sustained-release theophylline formulation in adult asthmatics. *Drug Dev Ind Pharm*. 1999;25:529-534.

66. Glynn-Barnhart A, Hill M, Szefler SJ. Sustained release theophylline preparations. Practical recommendations for prescribing and therapeutic drug monitoring. *Drugs*. 1988;35:711-726.

67. Hook GE, Bend JR. Pulmonary metabolism of xenobiotics. *Life Sci*. 1976;18:279-290.

68. Robertson CS, Gopinath SP, Uzura M, Valadka AB, Goodman JC. Metabolic changes in the brain during transient ischemia measured with microdialysis. *Neurol Res*. 1998;20:S91-S94.

69. Frykholm P, Hillered L, Langstrom B, et al. Increase of interstitial glycerol reflects the degree of ischaemic brain damage: a PET and microdialysis study in a middle cerebral artery occlusion-reperfusion primate model. *J Neurol Neurosurg Psychiatry*. 2001;71:455-461.

70. Parkin M, Hopwood S, Jones DA, et al. Dynamic changes in brain glucose and lactate in pericontusional areas of the human cerebral cortex, monitored with rapid sampling on-line microdialysis: relationship with depolarisation-like events. *J Cereb Blood Flow Metab*. 2005;25:402-413.

71. Taggart DP, el-Fiky M, Carter R, Bowman A, Wheatley DJ. Respiratory dysfunction after uncomplicated cardiopulmonary bypass. *Ann Thorac Surg*. 1993;56:1123-1128.

72. Theroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) Investigators. *Circulation*. 2000;102:3032-3038.

73. Afzelius BA. A human syndrome caused by immotile cilia. *Science*. 1976;193:317-319.

74. Robinson M, Bye PT. Mucociliary clearance in cystic fibrosis. *Pediatr Pulmonol*. 2002;33:293-306.